activity for the array of ESTs such that another non-asserted utility would be well established for the compounds.

This rejection is respectfully traversed.

Applicants have asserted in previous Amendments of August 23, 2002 and May 7, 2003, that the claimed method using the combination of *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401 is supported by a substantial patentable utility.

"Substantial utility" is defined in part in the REVISED INTERIM UTILITY GUIDELINES TRAINING MATERIALS, http://www.uspto.gov/web/menu/utility.pdf as follows:

> "Substantial utility" – A utility that defines a "real world" use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring. ...

Applicants submitted a Declaration by Dr. Randy Berka in the Amendment of September 22, 2003, where Dr. Berka disagreed with the Office Action's contention that the "claimed combination of nucleic acids is not supported by a substantial utility" on the ground that "[n]o evidence has been disclosed that the elected SEQ ID NOS allow for determination of the state or type of cell that is assayed by the claimed method of using an array of *Aspergillus oryzae* ESTs."

"Substantial utility" requires a utility that defines a "real world" use. The study described by Dr. Berka in his declaration provides a utility for the *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401 that meets the definition of "substantial utility."

In the art of industrial biotechnology, knowledge of the cellular processes and biochemical pathways that operate during a fermentation of a microorganism to produce a product of interest is of primary importance in developing a commercially successful fermentation process. The methods of the claimed invention involving the *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401 can provide such information so the skilled artisan would know how to cultivate a microorganism to develop a commercially successful fermentation process.

Dr. Berka described the use of a microarray of *Aspergillus oryzae* ESTs of the instant invention to study a recombinant *Aspergillus oryzae* strain (Le-1) containing a *Thermomyces lanuginosus* lipase gene and a yield- and morphologically-improved mutant (7-1) of Le-1 to understand why the recombinant strain Le-1 displayed a very pronounced "ballooning" cell state when grown under fermentation conditions and after approximately 90 hours of fermentation

production of the lipase ceased, while mutant 7-1 displayed a much lower degree of ballooning and did not stop producing lipase after 90 hours. The "ballooning" was detrimental to achieving a commercially successful fermentation process with recombinant strain Le-1 because of lipase production ceasing after approximately 90 hours of fermentation. An understanding of the genes that contributed to the "ballooning" cell state would provide information on how to cultivate a microorganism to develop a commercially successful fermentation process.

Dr. Berka indicated that analysis of the microarrays revealed a number of genes whose transcript levels differed between Le-1 and 7-1. Samples of mRNA extracted from Le-1 and 7-1 cultivated in 1 L fermentors for 3 or 4 days were labeled and hybridized to a microarray containing *Aspergillus oryzae* ESTs. Among these genes were 53 ribosomal genes and 27 genes identified as being involved in cell wall synthesis and morphogenesis. The difference in the transcript levels of the 27 genes between Le-1 and 7-1 indicated that general protein production and cell wall metabolism was affected differently in the strains, which was consistent with the observation that the morphology of the two strains was different, especially with regard to the degree of "ballooning" when expressing the lipase gene.

Dr. Berka also indicated that the 53 ribosomal genes were consistently down-regulated in 7-1 compared to Le-1 on both day 3 and day 4 of the fermentation and that the consistent down-regulation of genes involved in protein synthesis indicated that 7-1 had generally reduced translational activity compared to Le-1. He also indicated that such a reduction in translational activity would relieve some of the secretional stress and reduce the degree of ballooning seen in Le-1. He further indicated that the down-regulation reduced the counter-selective pressure against lipase-producing cells that were probably responsible for the rapid loss of expression potential in Le-1 cultures after 90 hours of fermentation, allowing 7-1 to retain its expression potential throughout the fermentation.

Dr. Berka also disclosed that 27 genes involved in cell-wall synthesis were differentially regulated where twenty of these genes were consistently up-regulated while seven were down-regulated in 7-1 compared to Le-1. The results indicated that the β-glucan synthesis pathway was up-regulated in 7-1 compared to Le-1. The physiological effect of the increased activity of this pathway was consistent with the reduced ballooning seen in 7-1, indicating that the ballooning cell state was likely caused by a deficiency of cell-wall components at the hyphae elongation sites, and that this deficiency would result in immense overloading of the secretion machinery in the cells. The results obtained with 7-1 compared to Le-1 led to a solution of what was causing the ballooning: While the up-regulation of proteins that take part in cell-wall synthesis *in situ* does not reduce the pressure on the secretion pathway, it increases the secretion of cell-wall synthesis enzymes at the

expense of other, less crucial, enzymes, *e.g.*, *Thermomyces lanuginosus* lipase.

Dr. Berka concluded that the results clearly demonstrated that the microarrays containing *Aspergillus oryzae* ESTs of the instant invention provided a powerful tool to study the effect of strain differences on global gene expression in the cells, from ballooning and non-ballooning states. He further concluded that without the claimed method it would have been essentially an impossible task to identify the pathways and ideally the genes that caused the ballooning resulting in the overloading of the secretion machinery in the cells and loss of lipase production.

The results of the study provide an example of a real world use for the *Aspergillus oryzae* ESTs in solving a problem. Applicants have asserted in the record that the use of an array of the *Aspergillus oryzae* ESTs enables a determination of the expression profiles of a plurality of genes that correlate, for example, with adaptation to changes in culture conditions, environmental stress, or other physiological provocation of a filamentous fungal cell of interest. For example, the expression profile data can provide information on individual transcript levels for biochemical pathways, transcription, translation, membrane cell wall synthesis and other cellular processes which leads to the capability to understand and solve problems that occur in the real world of industrial biotechnology.

The microarray of *Aspergillus oryzae* ESTs allowed the measurement of individual transcript levels of *Aspergillus oryzae* strains which correlated to a predisposition to the onset of a particular cell state, *i.e.,* "ballooning," that was detrimental to achieving a commercially successful fermentation process, and led to the identification of genes that contribute to the "ballooning" cell state for diagnosis, prevention, or further monitoring. The information provided led to a simple solution to preventing the onset of the ballooning cell state by changing the medium composition. Moreover, the results of the study provided an explanation of why mutant 7-1 retained its lipase expression potential throughout a fermentation while strain Le-1 did not.

Finally, the Office Action stated: "[I]t is not apparent that the data in the example detailed in the declaration comprises the probes of SEQ ID NO: 4377-7401 to which the claimed invention is limited. For example, a review of Table 3 of the specification does not reveal description of probe TREMBL Q96WT5 of the declaration. As such it cannot be determined whether the data of the declaration supports a utility of the claimed invention."

The ESTs used in the study described in Dr. Berka's declaration were *Aspergillus oryzae* ESTs disclosed in the instant application. For example, TREMBL Q96WT5 corresponds to SEQ ID NO: 5814.

Applicants assert, therefore, that the claimed method using the combination of nucleic acids of *Aspergillus oryzae* ESTs is supported by a substantial patentable utility. For the foregoing

reasons, Applicants submit that the rejection under 35 U.S.C. § 101 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 103-110 under 35 U.S.C. § 112, First Paragraph

Claims 103-110 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that one skilled in the art would not know how to use the claimed invention since it is not supported by a substantial utility or a well established utility. This rejection is respectfully traversed.

Based on Applicants' arguments in Section I, Applicants assert that one skilled in the art would know how to use the claimed invention because it is supported by a substantial utility.

For the foregoing reason, Applicants submit that the rejection under 35 U.S.C. § 112, first paragraph, has been overcome and respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 103-110 under 35 U.S.C. § 112, Second Paragraph

Claims 103-110 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite because they are not limited to a combination consisting of SEQ ID NO: 4377-740, which is the elected combination of sequences. This rejection is respectfully traversed.

The Office Action of February 25, 2002 requested that Applicants affirm the election, made during a telephone conversation on 24 January 2002, to prosecute the invention of Invention 1 of claims 1, 2, 11, 19, 20, 34, and 40 and the species *Aspergillus oryzae* EST combination of SEQ ID NOS: 4377-7401. In the Amendment of August 23, 2002, Applicants confirmed the election of claims 1, 2, 11, 19, 20, 34 and 40 and the species *Aspergillus oryzae* and corresponding EST combination of SEQ ID NOS: 4377- 7401. The Office Action of November 7, 2002 stated: "The election of the applicants of SEQ ID NOS: 4377-7401 as the species of probes satisfies the species requirement."

Applicants submit that the record clearly establishes that the species *Aspergillus oryzae* EST combination is SEQ ID NOS: 4377-7401.

For the foregoing reason, Applicants submit that the rejection under 35 U.S.C. § 112, second paragraph, has been overcome and respectfully request reconsideration and withdrawal of the rejection.

IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the

undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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